Microbubbles coated with anti-P-selectin antibody RB40.34
MB-RB40.34

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Chemical name:	Microbubbles coated with anti-P-selectin antibody RB40.34
Abbreviated name:	MB-RB40.34, MBp
Synonym:	
Backbone:	Antibody
Target:	P-selectin
Mechanism:	Antibody-antigen binding
Method of detection:	Ultrasound
Source of signal:	Microbubbles
Activation:	No
Studies:	✓ *In vitro* ✓ Rodents

Click on protein [http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=200553], nucleotide [http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=6031196] (RefSeq), and gene for more information about P-selectin.

Background

[PubMed]

Ultrasound is the most widely used imaging modality (1) and its role in noninvasive molecular imaging with ligand-carrying microbubbles is expanding (2). Microbubbles are spherical cavities filled by a gas encapsulated in a shell. The shells are made of phospholipids, surfactant, denatured human serum albumin, or synthetic polymer. Ligands and antibodies can be incorporated into the shell surface of microbubbles. Microbubbles are usually 2 to 8 μm in size. They provide a strongly reflective interface and resonate to ultrasound waves. They are used as ultrasound contrast agents in imaging of inflammation, angiogenesis, intravascular thrombus, and tumors (3-5). They may also potentially be used for drug and gene delivery (6).

Endothelial cells are important cells in inflammatory responses (7, 8). Bacterial lipopolysaccharide, virus, inflammation, and tissue injury increase tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and secretion of other cytokines and chemokines. Leukocyte emigration from blood is dependent on rolling of leukocytes along endothelial cell surfaces and subsequent adherence to endothelial cell surfaces. Inflammatory mediators and cytokines induce chemokine secretion from endothelial cells and other vascular cells and increase their expression of cell surface adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1

(VCAM-1), integrins, and selectins. Chemokines are chemotactic to leukocytes to sites of inflammation and tissue injury. The movement of leukocytes through endothelial junctions into the extravascular space is highly orchestrated through various interactions with different adhesion molecules on endothelial cells (9).

P-selectin is found on the cell surface of endothelial cells and platelets (8, 10). It binds to glycoprotein on the cell-surface of leukocytes. IL-1 and TNFα, released from inflammatory and ischemic stimuli, stimulate P-selectin and other adhesion molecule expression on the vascular endothelial cells leading to leukocyte adhesion to the activated endothelium. P-selectin and other selectins are involved in rolling and arresting leukocytes on the endothelium. Microbubbles targeted with antibodies against P-selectin are being developed as noninvasive agents for P-selectin expression in vascular endothelial cells of inflamed and damaged tissues (11).

Synthesis

[PubMed]

For targeted microbubbles, biotinylated microbubbles were first prepared by sonication of an aqueous dispersion of decafluorobutane gas, distearoylphosphatidylcholine, polyethyleneglycol (PEG)- stearate, and distearoylphosphatidylethanolamine-PEG-biotin (11). Microbubbles were combined with streptavidin, washed, and conjugated with biotinylated monoclonal antibody (RB40.34) against P-selectin (MB_p) or isotype-control monoclonal antibody (R3-34) against α_v integrin (MB_{iso}). Control lipid microbubbles (MB_c) were also prepared. The microbubbles are about 3.5 ± 1.4 μm in diameter. An antibody to microbubble ratio was estimated to be 102 ± 3 x 10^3 by flow cytometry.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Takalkar et al. (12) reported that MB_p (5 x 10^6 particles/ml) perfused through the flow chamber coated with P-selectin (109 sites/μm^2) at a shear stress of 0.3 dyn/cm^2 accumulated at a rate of 565 mm^{-2} min^{-1}. Attachment rates increased at higher plate surface densities of P-selectin and MB_p detachment was reduced. Accumulation rate first increased with shear, reached a maximum stress force at ~0.6 dyn/cm^2, and then decreased. Minimal microbubble attachment was observed on a plate that lacked P-selectin or was blocked with RB40.34. Microbubble detachment was tested by stepping up shear stress at 30-s intervals. Half-maximal detachment was reached at 34 dyn/cm^2. Therefore, accumulation and retention of MB_p is possible under physiologic flow conditions and is strongly influenced by shear stress and surface density of the target receptor. Furthermore, Rychak et al. (13) demonstrated that deformed MB_p exhibited significantly greater attachment efficiency than spherical MB_p at stress forces of 0.4–1.35 dyn/cm^2.

Animal Studies

Rodents

[PubMed]

Lindner et al. (11) performed ultrasound assessment of inflammation with MB_p by intravital microscopy of the cremasteric venules of control (n = 5) and TNFα–stimulated wild-type mice (n = 5). Retention of MB_p, MB_{iso}, and MB_c increased (P<0.05) with TNFα treatment because of increased attachment to activated leukocytes. Extensive attachment of MB_p directly to the endothelium was observed in TNFα–stimulated mice, resulting in retention of MB_p (16 microbubbles/measurement) >3-fold (*P*<0.01) greater than either MB_{iso} (4 microbubbles/measurement) or MB_c (2 microbubbles/measurement). Enhanced retention of MB_p was completely abolished in TNFα–stimulated P-selectin–deficient mice. Renal ultrasound imaging of the kidneys of wild-type mice (n = 5) undergoing ischemia-reperfusion injury showed that video intensity signal was significantly higher (*P*<0.05) for MB_p (12 ± 2 U) than either MB_{iso} (6 ± 3 U) or MB_c (5 ± 3 U). In P-selectin-deficient mice (n = 3), the signal for MB_p was equivalent to that from control microbubbles.

Rychak et al. (13) demonstrated that deformed MB_p exhibited significantly greater attachment than spherical MB_p in the cremasteric venules of TNFα–treated wild-type mice (*P*<0.05, n = 4). There was no difference in adhesion of deformed MB_p and spherical MB_p in the cremasteric venules of TNFα–treated P-selectin–deficient mice (n = 3); rather, the adhesion measurements were 6- and 2-fold lower, respectively.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

References

1. Wells PN. Physics and engineering: milestones in medicine. Med Eng Phys 23(3):147–153; 2001. (PubMed)
2. Liang H.D., M.J., Blomley . The role of ultrasound in molecular imaging. Br J Radiol 76 Spec No 2:S140–S150; 2003. (PubMed)
3. Klibanov AL. Ligand-carrying gas-filled microbubbles: ultrasound contrast agents for targeted molecular imaging. Bioconjug Chem 16(1):9–17; 2005. (PubMed)

4. Lindner JR. Microbubbles in medical imaging: current applications and future directions. Nat Rev Drug Discov 3(6):527–532; 2004. (PubMed)

5. Villanueva FS, Wagner WR, Vannan MA, Narula J. Targeted ultrasound imaging using microbubbles. Cardiol Clin 22(2):283–298; 2004. (PubMed)

6. Dijkmans PA, Juffermans LJ, Musters RJ, van Wamel A, ten Cate FJ, van Gilst W, Visser CA, de Jong N, Kamp O. Microbubbles and ultrasound: from diagnosis to therapy. Eur J Echocardiogr 5(4):245–256; 2004. (PubMed)

7. Cybulsky MI, Gimbrone MA. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science 251(4995):788–791; 1991. (PubMed)

8. Lowe JB. Glycosylation in the control of selectin counter-receptor structure and function. Immunol Rev 186:19–36; 2002. (PubMed)

9. Vanderslice P, Woodside DG. Integrin antagonists as therapeutics for inflammatory diseases. Expert Opin Investig Drugs 15(10):1235–1255; 2006. (PubMed)

10. Chen M, Geng JG. P-selectin mediates adhesion of leukocytes, platelets, and cancer cells in inflammation, thrombosis, and cancer growth and metastasis Arch Immunol Ther Exp (Warsz) 2006.

11. Lindner JR, Song J, Christiansen J, Klibanov AL, Xu F, Ley K. Ultrasound assessment of inflammation and renal tissue injury with microbubbles targeted to P-selectin. Circulation 104(17):2107–2112; 2001. (PubMed)

12. Takalkar AM, Klibanov AL, Rychak JJ, Lindner JR, Ley K. Binding and detachment dynamics of microbubbles targeted to P-selectin under controlled shear flow. J Control Release 96(3):473–482; 2004. (PubMed)

13. Rychak JJ, Lindner JR, Ley K, Klibanov AL. Deformable gas-filled microbubbles targeted to P-selectin. J Control Release 114(3):288–299; 2006. (PubMed)